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Antiangiogenic agent

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Detailed Description

1. Title of the Invention

Antiangiogenic agent

2. Patent Claims

(1) An antiangiogenic agent, characterized by the fact that it contains a substance with anti-inflammatory activity as the major component.

3. Detailed Description of the Invention

The present invention relates to an antiangiogenic agent. More specifically, the present invention relates to an anti-angiogenic agent containing a substance with anti-inflammatory activity as the major component.

For animals, in particular animals with a closed circulation system, blood vessels are essential for supporting life. They transport nutrients to tissues and organs and metabolic waste from tissues and organs, playing an important physiological role. The structures, maintenance and improvements of the functions of blood vessels have been extensively studied, focused on disease conditions such as hypertension, ischemic disorders, etc. Recently, it was found that the physiologically very important blood vessels sometimes exhibit abnormal growth, resulting in induction or worsening of various diseases. For example, in some diabetes patients, abnormal growth of blood capillaries occurs in the vitreous body that may result in loss of sight. For cancers that have become the No. 1 cause of death in our country, it has been known that there is abnormal angiogenesis from the host to the tumor tissue (J. Folkman, Separate Volume of Science, pages 85-97, Nikkei Science Publisher, 1981). If there is no such abnormal growth of host blood vessels, solid tumors cannot grow larger than several millimeters (diameter) and the tumor cannot become lethal.

The inventors reasoned that if abnormal angiogenesis can be prevented or suppressed, various diseases may be prevented or treated; and carried out active studies. As a result, they found that antiinflammatory agents (in the following, simply called the agent) have not only the functions of fever relief, analgesia and antiinflammatory action, but also exhibit inhibitory activity against angiogenesis. The present invention has thus been achieved.

Thus far, there has been no patent describing that diseases can be prevented or treated from this standpoint. Thus, it can be said that the present invention is a breakthrough in providing prevention and treatment agents.

The agent can be a steroidal or nonsteroidal drug. The following are some examples.

Steroidal drugs: cortisone acetate, hydrocortisone acetate, prednisolone, dexamethasone, dexamethasone sodium phosphate.

Nonsteroidal drugs:

Aniline derivatives: acetanilide, acetaminophen, phenacetin, lactyl phenetidine, bucetin, phenylacetylglycine dimethylamide, bromanil promide (?).

Salicylic acid derivatives: salicylic acid, sodium salicylate, calcium salicylate, lithium salicylate, aspirin, aspirin aluminum, choline salicylate, methyl salicylate, salicylic amide, ethoxybenzamide.

Quinophene derivatives: quinine ethyl carbonate, quinine hydrochloride, quinine sulfate, quinophene.

Pyrazolone derivatives: aminopyrine, sulpyrin, aminopropylon, antipyrine, isopropyl antipyrine, migrenin [?], phenopyrazone, nifenazone, phenylbutazone, oxyphenbutazone, ketophenylbutazone, chlophezone [?], azapropazone, sulfinpyrazone, difenamizole.

Anthranilic acid derivatives: mefenamic acid, aluminum flufenamate, flufenamic acid, niflumic acid, glafenine.

Phenylacetic acid derivatives: ibuprofen, alclofenac, ketoprofen, naproxen, flubiprofen [?], fenbufen, ibufenac, diclofenac sodium, fentiazac.

Pyrimidine derivatives: bucolome, mepirizole.

Phenothiazine derivatives:: metiazinic acid, protizinic acid, dimethothiazine.

Indole acetate derivatives:: indomethacin, tolmetin sodium, sulindac, clidanac.

Basic substances: tinoridine hydrochloride, tialamide hydrochloride, perisoxal citrate, benzydamine hydrochloride.

Others: tripenoxide (?), benfepazone (?), phenylamidole [?] hydrochloride, simetride, diphenyldimethylaminoethane hydrochloride, ethoheptazine, pentazocine, azabicyclan, propoxyphene hydrochloride, propoxyphene napsilate (?), colchicine, benzbromarone, probenecid, imipramine, carbamazepine, bumadizon, piroxicam, fenoprofen calcium, planoprofen (?), sasapyrine (?), and feprazone.

These drugs are described in Therapeutic Agent Handbook, New Comprehensive Version (Yakugyo Jiho Publisher, published on May 15, 1979), pages 70-101; Sogo Rinsho, vol. 30, Supplement Issue (Prescription Planning), pages 55-60 and 178-180, 1981; Sogo Rinsho, vol. 39, Supplement Issue, pages 156-160, 1981; Japanese Therapeutic Agents Collection (5th Version), Yakugyo Jiho Publisher (1979); Drug Name Indexing Dictionary, Yakugyo Jiho Publisher (1981); etc.

The inhibitory effect of the agent on angiogenesis was investigated as described in "Medical Progresses" vol. 122, page 890, 1982, and it was confirmed that the agent could nicely suppress abnormal growth of blood vessels in tumor tissues of experimental animals. Moreover, it has been confirmed that in original tumors and metastatic locations angiogenesis was suppressed in cancer patients. In addition, in proliferative omentitis and diabetic omentitis, it was confirmed that the abnormal growth of blood capillaries in the vitreous body could be suppressed.

As described above, the agent has antiangiogenic activity, and is effective in the prevention or treatment of proliferative omentitis, rheumatoid arthritis, chronic eczema, diabetic omentitis, immature omentitis, tumors, etc.

When the agent is used as an antiangiogenic agent, it is administered at a dose sufficient for obtaining the therapeutic effect which is dependent on the particular disease. For oral administration, its dose form can be powder, grain, tablet, controlled release form,

sugar-coated tablet, capsule, syrup, pill, suspension, liquid, emulsion, etc. For injection, the agent can be prepared in ampules, bottles, etc. Suppository and ointment also can be used.

The agent can be used by itself or in combination with pharmaceutically appropriate diluents or with other drugs. The diluent can be solid, liquid or semisolid excipient, binder, wetting agent, disintegrator, surface-active agent, lubricant, dispersing agent, buffer, perfume, preservative, dissolution assistant, solvent, etc.

When the agent is used in a dosage form, the active component in the dosage form is generally 0.01-100% (by weight), preferably 0.05-80% (by weight).

The agent can be administered to animals and humans orally or parenterally. The oral administration includes the sublingual route. The parenteral administration includes injections (such as subcutaneous, intramuscular, intravenous injections and intravenous drip), rectal administration, etc. It also can be used by coating.

The dose of the agent depends on animal species, age, disease, etc., and thus it may be out of the following ranges. In general, in humans, the dose of the agent is 0.1-1500 mg/kg per day, preferably 1-500 mg/kg. The dose can be divided and administered 2-4 times a day.

In the following, the present invention is further described in detail by the way of practical examples.

Practical Example 1

Suppression of abnormal angiogenesis in mouse sarcoma-180 tumor tissue and improvement of the tumor

Sarcoma-180 cancer cells (5×10^6) were sealed into Millipore Diffusion Chamber (PR000/401, from Millipore Japan) then planted into fascia at the back of 8 week old ICR mice (Medical Progresses, vol. 122, page 890, 1982). On day 9 after planting, host-mouse-derived abnormal angiogenesis was investigated. As shown in Fig. 1, significant abnormal angiogenesis was observed. By contrast, if tolmetin sodium was administered orally on days 3, 5 and 8 at 100 mg/kg per day, and then the animal was examined on day 9, as shown in

Fig. 2, no abnormal angiogenesis could be recognized. Thus, the inhibitory effect on abnormal angiogenesis was confirmed.

Based on these results, 10^6 of Sarcoma-180 cancer cells were planted subcutaneously in ICR mouse and, on days 3, 5 and 8 after planting, tolmetin sodium was administered orally at 100 mg/kg per day. In the control group, on day 3, 5 and 8 after planting, saline was administered orally.

Table 1 shows the results of tumor growth inhibition rates calculated from the average tumor weights on day 25 after planting (average from 5 animals in each group). As it can be clearly seen in Table 1, compared to the control group, the tolmetin sodium group showed a tumor growth inhibition rate of 32.0%, demonstrating that tolmetin sodium has significant effect on improvement of the tumor.

Table 1

	average tumor weight (g)	inhibition rate (%)
control group	4.344 ± 1.889	0
drug group	2.952 ± 1.167	32.0

In addition, oral administration of phenylacetylglycine dimethylamide at 500 mg/kg, sodium salicylate at 500 mg/kg, aspirin aluminum at 1 g/kg, choline salicylate at 800 mg/kg, salicylic amide at 500 mg/kg, aminopyrine at 100 mg/kg, sulpyrin at 1 g/kg, phenylbutazone at 200 mg/kg, mefenamic acid at 500 mg/kg, aluminum flufenamate at 500 mg/kg, flufenamic acid at 200 mg/kg or diclofenac sodium at 50 mg/kg also resulted in the inhibition of the abnormal angiogenesis and the improvement of the tumor (inhibition of tumor growth).

Practical Example 2

Suppression of abnormal angiogenesis in mouse sarcoma-180 tumor tissue and improvement of the tumor

Sarcoma-180 cancer cells (5×10^6) were sealed into Millipore Diffusion Chamber (PR000/401, from Millipore Japan) then planted into fascia at the back of 8 weeks old ICR mouse (Medical Progresses, vol. 122, page 890, 1982). On day 9 after planting, the host-mouse-derived abnormal angiogenesis was investigated. As shown in Fig. 3, significant

abnormal angiogenesis was observed. By contrast, if hydrocortisone acetate was administered orally on day 3, 5 and 8 at 15 mg/kg per day, and then the animal was examined on day 9, as shown in Fig. 4, no abnormal angiogenesis could be recognized. Thus, the inhibitory effect on abnormal angiogenesis was confirmed.

Based on these results, 10^6 of Sarcoma-180 cancer cells were planted subcutaneously in ICR mouse and, on day 3, 5 and 8 after planting, hydrocortisone acetate was administered intramuscularly at 15 mg/kg per day. In the control group, on day 3, 5 and 8 after planting, saline was administered intramuscularly.

The results of tumor growth inhibition rate calculated from the average tumor weights on day 25 after planting (average from 5 animals in each group) showed that, compared to the control group, the hydrocortisone acetate group had a tumor growth inhibition rate of about 35%, demonstrating that hydrocortisone acetate has significant effect on improvement of the tumor.

In addition, oral administration of metiazinic acid at 250 mg/kg, glafenine at 1 g/kg, benzydamine hydrochloride at 150 mg/ml, bucolome at 500 mg/kg, mepirizole at 150 mg/kg, azapropazone at 500 mg/kg, tinoridine hydrochloride at 500 mg/kg, chlophezone (?) at 500 mg/kg, sulindac at 200 mg/kg, naproxen at 300 mg/kg or piroxicam at 100 mg/kg also resulted in the inhibition of the abnormal angiogenesis and the improvement of the tumor (inhibition of tumor growth).

In addition, when mouse liver cancer MH-134, Lewis' lung cancer cells, rat carcinoma or Walker 256 cells were used as the tumor cells, and C3H/He mice, BDF_1 mice or Wistar rats as the experimental animals were used, the same results were obtained.

Practical Example 3

Suppression of Lewis' lung cancer cells-derived abnormal angiogenesis in BDF_1 mice and improvement of the tumor

Based on Practical Example 1, Lewis' lung cancer cells (10^6) were planted subcutaneously in BDF_1 mouse, and inhibition of the abnormal angiogenesis and improvement of the tumor were investigated. It was found that by oral administration of tolmetin sodium at 100 mg/kg per day the tumor was significantly improved. Table 2 shows the survival rates (%) calculated based of survival days (average from 5 animals in each group).

Table 2

	survival days (day)	survival rate (%)
control group*	21.2 ± 6.2	100
drug group	27.8 ± 8.1	131.2

*The group to which saline alone was administered.

Similarly, oral administration of hydrocortisone acetate at 18 mg/kg or aspirin at 160 mg/kg resulted in survival rates of 134.1% and 130.1%, respectively.

In addition, oral administration of glafenine at 1 g/kg, clidanac (?) at 250 mg/kg, ketoprofen at 150 mg/kg, fenoprofen calcium at 250 mg/kg, fentiazac at 200 mg/kg, fenbufen at 350 mg/kg, planoprofen (?) at 150 mg/kg, flubiprofen (?) at 200 mg/kg, prothiazinic (?) acid at 500 mg/kg, alclofenac at 500 mg/kg or feprazone at 1.5 g/kg also resulted in the inhibition of the abnormal angiogenesis and the improvement of the tumor.

Practical Example 4

Effect on experimental diabetic omentitis caused in Wistar rats

In 5 weeks old male Wistar rat streptozotocin was injected intravenously at the tail at 65 mg/kg. After about 3 months, 3,3'-iminodipropionitrile was administered to induce experimental diabetic omentitis. In some of the rats there appeared abnormal growth of blood capillaries in the vitreous body (the so-called proliferative omentitis). Rats with the proliferative omentitis were selected, and 3,3'-iminodipropionitrile was administered, followed by administration of indomethacin at 2.5 mg/kg per day, every other day. In such a group, from day 3 after the administration, the abnormal growth of blood capillaries in the vitreous body was significantly suppressed, thereby confirming that the agent has improving effect on diabetic omentitis. Similarly, by administration of aspirin at 200 mg/kg per day, improvement of the disease condition was observed.

Practical Example 5

Improving effect on tumors in tumor patients

Tolmetin sodium was administered at 500 mg/day in a liver cancer patient (stage IV, 57 years old, male) who could not undergo surgery due to various reasons, and the effect on improvement of the cancer was examined. It was found that good improvement of the cancer was achieved over a long time period.

Practical Example 6

Improving effect on diabetic omentitis in patients

In addition to cyclopidine (?), aspirin was administered at 1.5 g/kg in a long time diabetes patient (54 years old, male) who had progressing growth of blood capillaries in the vitreous body, and it was confirmed by using funduscopy that the abnormal growth of blood capillaries, that could not be suppressed by protective therapy, was inhibited. Furthermore, the progress of the disease could be completely suppressed by combination of the agent and phototherapy.

Practical Example 7

Improving effect on diabetic omentitis in patients

In addition to cyclopidine (?), prednisolone was administered at 1 mg/day in a long time diabetes patient (49 years old, male) who had progressing growth of blood capillaries in the vitreous body, and the abnormal growth of blood capillaries was thus inhibited. Furthermore, the progress of the disease could be completely suppressed by combination of the agent and phototherapy.

Pharmaceutical Preparation Example 1

1.5 part (by weight) of aspirin, 8.0 parts (by weight) of simple syrup and 100 parts (by weight) of purified water were used to prepare an oral dose form.

Pharmaceutical Preparation Example 2

40 mg of dexamethasone sodium phosphate was added to sterilized saline to prepare an injection in 10 ml.

The acute toxicity values for typical agents are listed below. The acute toxicity value was measured in mice by oral administration, except that aspirin was in rats by oral administration and prednisolone was in mice by subcutaneous injection.

Table 3

name of the agent	LD_{50}(mg/kg)
azapropazone	1400
aspirin	1750
aspirin aluminum	3700
aminopyrine	438
alclofenac	1630
ibuprofen	900
indomethacin	50
tinoridine hydrochloride	2050
benzydamine hydrochloride	540
oxyphenbutazone	480
glafenine	3500
clidanac(?)	825
chlophezone (?)	2140
ketoprofen	560
choline salicylate	2690
salicylic amide	1400
sodium salicylate	1600
diclofenac sodium	145
sulindac	621
sulpyrin	3500
thialamide (?)	564
tolmetin sodium	1380
naproxen	991
piroxicam	350
phenylacetylglycine dimethylamide	1600
phenylbutazone	680
fenoprofen calcium	757
feprazone	5150
fentiazac	620
fenbufen	1050
bucolome	1550
planoprofen (?)	466
flufenamic acid	708
aluminum flufenamate	1460
flubiprofen	800
prednisolone	)3500
protiazinic (?) acid	1518
metiazinic acid	870
mepirizole	1022
mefenamic acid	1413

4. Brief Legends to the Figures

Fig. 1 and Fig. 3 show the abnormal angiogenesis in the control groups, while Fig. 2 and Fig. 4 show the inhibition of the abnormal angiogenesis in the groups of the present invention.

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Fig. 1

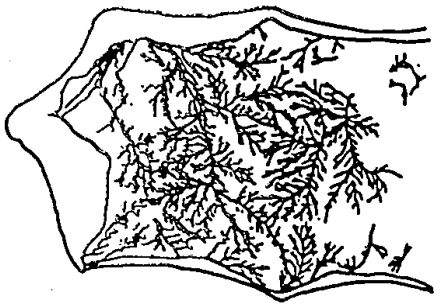

Fig. 2

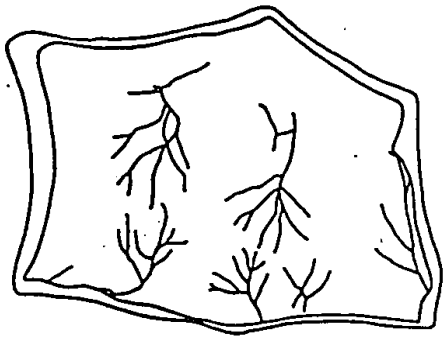

Fig. 3

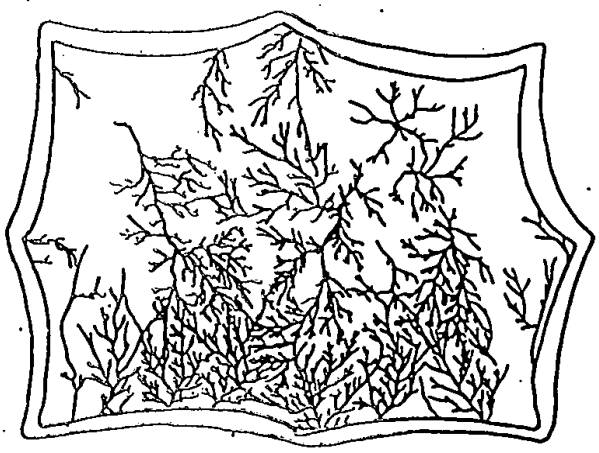

Fig. 4